REMARKS/ARGUMENTS

The Pending Claims

Claims 8-12 are pending and are directed to a method of producing a rat embryonic stem (ES) cell.

Amendments to the Claims

The claims have been amended to point out more particularly and claim more distinctly the invention. In particular, claim 8 has been amended to incorporate the features of claims 13 and 35 (now canceled). Additionally, claim 8 has been amended to recite that the method consists essentially of (instead of comprises) steps (A)-(E). The amendments to claim 8 are supported by the specification at, for example, page 17, lines 16-24.

Accordingly, no new matter has been added by way of these amendments to the claims.

Summary of the Office Action

The Office rejects claims 8, 10, 12, and 35 under 35 U.S.C. § 102(b) as allegedly anticipated by Loring (WO 99/27076).

The Office rejects claims 8 and 13 under 35 U.S.C. § 103(a) as allegedly obvious over Loring in view of Takahama et al. (*Oncogene, 16*: 3189-3196 (1998)). The Office rejects claims 8 and 9 under 35 U.S.C. § 103(a) as allegedly obvious over Loring in view of Price et al. (WO 98/30679). The Office rejects claims 8 and 11 under 35 U.S.C. § 103(a) as allegedly obvious over Loring in view of Vassilieva et al. (*Experimental Cell Research, 258*: 361-373 (2000)) and Mandalam et al. (U.S. Patent 7,297,539).

Reconsideration of these rejections is hereby requested.

Discussion of the Anticipation Rejection

The Office contends that the subject matter of claims 8, 10, 12, and 35 is anticipated by Loring. Applicants note that claim 35 has been canceled. Claim 8 (from which claims 10

and 12 depend) has been amended to incorporate the features of claim 13, which claim was not included in the anticipation rejection.

Since the pending claims, as amended, recite a feature that is not disclosed by Loring (i.e., that a rat LIF-containing medium is used in steps(C)-(E)), Loring cannot be considered to anticipate the pending claims. Therefore, Applicants request that the anticipation rejection be withdrawn.

Discussion of the Obviousness Rejections

The Office contends that the subject matter of one or more of claims 8, 9, 11, and 13 is obvious over Loring in view of one or more of Takahama et al., Price et al., Vassilieva et al., and Mandalam et al.. These rejections are traversed for the following reasons.

The pending claims, as amended, recite that the method of producing a rat embryonic stem cell consists essentially of steps (A)-(E), which are performed using a culture medium with 2% or less serum concentration: (A) culturing a rat blastocyst in a leukemia inhibitory factor (LIF)-free culture medium to form an inner cell mass in the blastocyst, (B) dissociating the inner cell mass, wherein the dissociated inner cell mass is in a cell aggregate state, (C) culturing primary embryonic stem cells resulting from a culture of the dissociated inner cell mass until the primary embryonic stem cells can be passaged, (D) dissociating the primary embryonic stem cells, which can be passaged, wherein the dissociated primary embryonic stem cells are in a cell aggregate state, and (E) culturing the dissociated primary embryonic stem cells to establish an embryonic stem cell, wherein a rat leukemia inhibitory factor (rLIF)-containing culture medium is used in steps (C)-(E).

Thus, the pending claims exclude essential steps other than steps (A)-(E), require that the steps are performed using a culture medium with 2% or less serum concentration, and require that a rLIF-containing culture medium is used in steps (C)-(E). None of the cited references teach or suggest the inventive methods when considered alone or in combination.

The Office notes that Loring teaches that rat ES cells were obtained only after co-culturing with mouse ES cells. As discussed above, the pending claims do not recite a step for co-culturing with mouse ES cells, and the claim language "consisting essentially of"

excludes essential steps other than steps (A)-(E). Loring, as well as the other cited references, do not teach or suggest that rat ES cells capable of producing a chimeric rat can be obtained without the co-culture with mouse ES cells by culturing rat blastocysts in LIF-free medium in the step for inducing the formation of inner cell mass (ICM), preferably, further performing the step for dissociating the ICM in LIF-free medium, and changing the culture medium to LIF-containing medium after primary ES cells emerge, as required by the pending claims.

The Office contends that Loring teaches the use of LIF-free medium during the culture inducing ICM formation in blastocysts (i.e., step (A) of claim 8) since the method of Loring comprises culturing harvested blastocysts or delayed blastocysts in any appropriate medium under any conditions which allow for growth and proliferation of ES cells and a medium for blastocyst culture exemplified in Loring does not contain LIF. However, one of ordinary skill in the art upon reading Loring (see, e.g., Example 2) would understand that the medium disclosed therein is a basal medium and that the culture media actually used for blastocyst culture are supplemented with (1) LIF, (2) LIF and SCF, or (3) LIF, SCF, and bFGF. Thus, all of the culture media used for blastocyst culture in Loring contain LIF, which conflicts with the steps of the inventive method.

The Office contends that Loring states that culture medium (i.e., (1) LIF, (2) LIF and SCF, or (3) LIF, SCF, and bFGF culture media) makes no apparent difference in the early blastocyst culture. However, this statement means that there is no difference between each of the three culture media (i.e., between (1) LIF, (2) LIF and SCF, and (3) LIF, SCF, and bFGF) rather than between LIF-free culture and (1) LIF, (2) LIF and SCF, or (3) LIF, SCF, and bFGF.

Additionally, in Example 5 of Loring, ES cell medium containing 2000 U of LIF is used for blastocyst culture. Accordingly, Loring teaches the use of LIF-containing medium rather than LIF-free medium for blastocyst culture as a whole. Applicants note that the phrase "ES cell medium (without LIF)" in Example 5 of Loring implies that ES cell medium other than that used for co-culture with mouse ES cells contains LIF.

The Office contends that Takahama et al. discloses the use of (i) IGF-II and (ii) IGF-II and LIF containing media for establishing ES/EC cells and that IGF-II is essential for the maintenance and growth of ES cells. Applicants note that in Takahama et al., LIF-containing medium always is used for establishing ES cell-like colonies. The effects of growth factors on the maintenance and proliferation of ESC-like cells are compared between LIF alone and LIF and IGF-II. As a result, LIF and IGF-II maintained ES cell phenotype, whereas LIF alone resulted in the differentiation. Thus, in Takahama et al., LIF always is present in culture media for the steps involving ICM formation in blastocysts to the steps involving the maintenance and proliferation of ESC-like cells.

Thus, based on the teachings of the cited references and, in particular, the Loring and Takahama references, one of ordinary skill in the art would believe that LIF was necessary for ICM formation in rat blastocysts. The Loring and Takahama references differ from the present invention in that they focus on the effect of rat LIF on the maintenance of stem cell phenotype of rat ES cells. The present invention is based on the finding that the use of LIF-free medium for blastocyst culture results in an efficient ICM formation in rat blastocysts and enables rat ES cell establishment without co-culture without ES cells. As discussed in the "Reply to Office Action" dated November 16, 2009, the superior effects resulting from the present invention were unexpected in view of the teachings of the prior art.

The Office contends that the previously submitted Rule 132 Declaration of Dr. Ochiya detailing the unexpected beneficial effects of the inventive method is irrelevant because Loring already taught the use of LIF-free medium for rat blastocyst culture. However, as discussed above, Loring teaches the use of LIF-containing medium for rat blastocyst culture as a whole. Therefore, the Rule 132 Declaration of Dr. Ochiya is relevant and provides further evidence of the non-obviousness of the inventive method.

The Office expresses concern about the alleged inconsistency between the data disclosed in the present invention and the data disclosed in Ueda et al., which is a post-filing reference by the inventors. In particular, the Office notes that the rat ES cells disclosed in Example 1 of the present invention are positive for alkaline phosphatase (see Fig. 13), while the rat ES cells disclosed in Ueda et al. are negative for alkaline phosphatase. Applicants note that the inconsistency likely is caused by the serum concentration contained in the

culture medium. In the examples of the present invention, the rat ES cells were cultured in serum-free medium. In contrast, the rat ES cells disclosed in Ueda et al. were cultured in 3% fetal bovine serum (FBS).

As discussed above, the pending claims, as amended, recite that the culture is substantially serum-free, which refers to a serum concentration of less than 2%. Thus, the culture method disclosed in Ueda et al., which uses a 3% FBS culture medium, is not encompassed by the claimed invention.

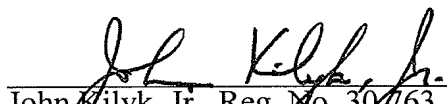
Applicants note that the critical evaluations in the Buehr and Li references cited by the Office apply to the rat ES cells of Ueda et al., which are distinct from the rat ES cells of the present invention. Applicants have provided evidence of the superior effects of the rat ES cells produced by the inventive methods in the Rule 132 Declaration of Dr. Ochiya.

Thus, for all of the above-described reasons, Applicants assert that the inventive methods are not obvious in view of the cited references, such that the obviousness rejections should be withdrawn.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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